Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information.

Mechanically robust photodegradable gelatin hydrogels for 3D cell culture and in situ mechanical modification

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Figure S1. Early attempts to produce photodegradable gelatin were unsuccessful. In these experiments, we attempted to directly attach the *o*-NB acrylate moiety (**1**) directly to the lysine primary amine using DMSO as a solvent. However, upon dialysis of the product, we found that the product crashed out of solution, and could not be dissolved, even at elevated temperatures or by using DMSO as the solvent.

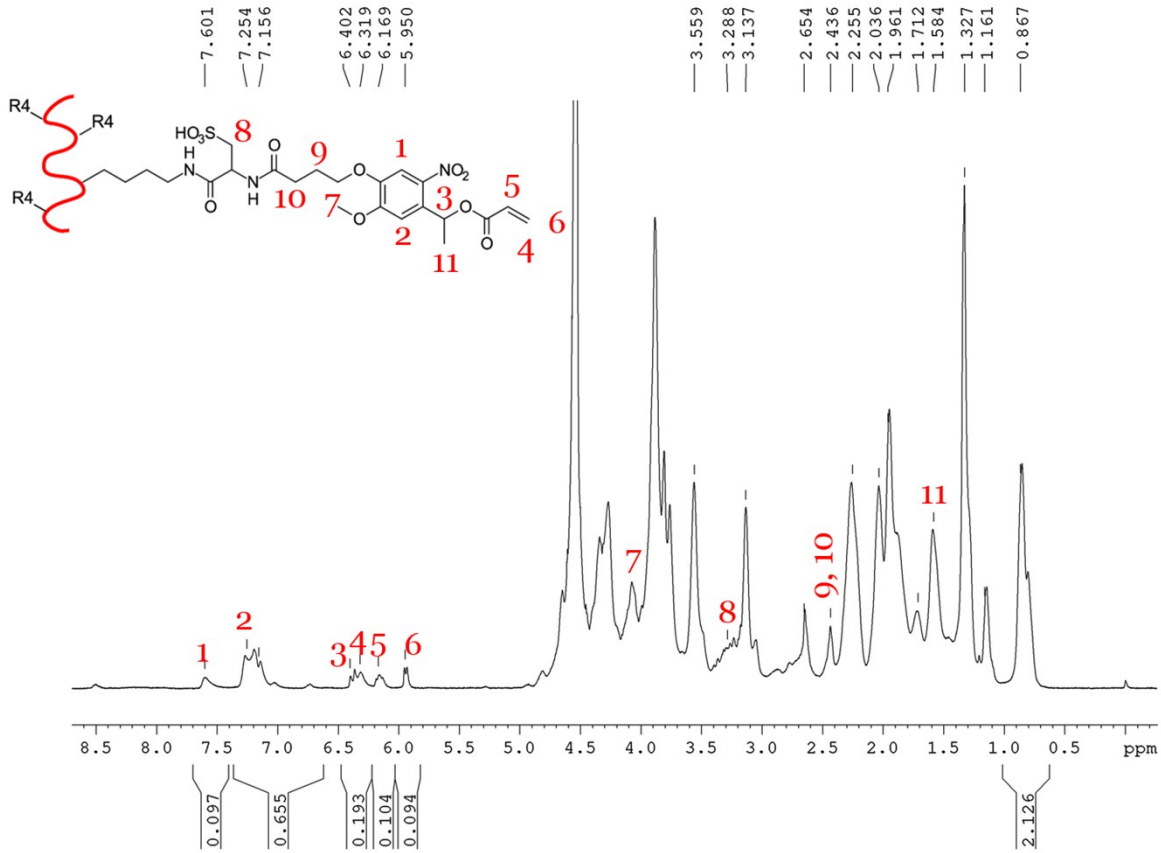

Figure S2. ^{1}H NMR Spectrum of acryl-oNB-sulfo-gelatin (10)

Synthesis Acryl-*o*NB-sulfo-gelatin (*o*NB-Gel) (10).
A mixture of **8** (433 mg, 0.0632 mmol primary amine) was dissolved in DMSO (20 mL) at 65° C. Once dissolved, the reaction mixture was brought down to 35° C. Compound **1** (76 mg, 0.168 mmol) was added and the reaction mixture was stirred overnight. The conjugation of the *o*-NB group to the gelatin was checked by TLC. The reaction mixture was precipitated in acetone (200 mL) and acidified with 2 M HCl until the gelatin was fully precipitated. The product was collected by centrifugation, washed twice with 20 mL acetone, dissolved in water, and then titrated with 0.1 M NaOH (aq.) till the solution reached pH=7.0. The solution was then dialyzed against water for 36 hours with 4 water changes and lyophilized to yield a colorless foam **10** (yield 427 mg, 90.7%, 0.098 mmol *o*-NB per gram gelatin).

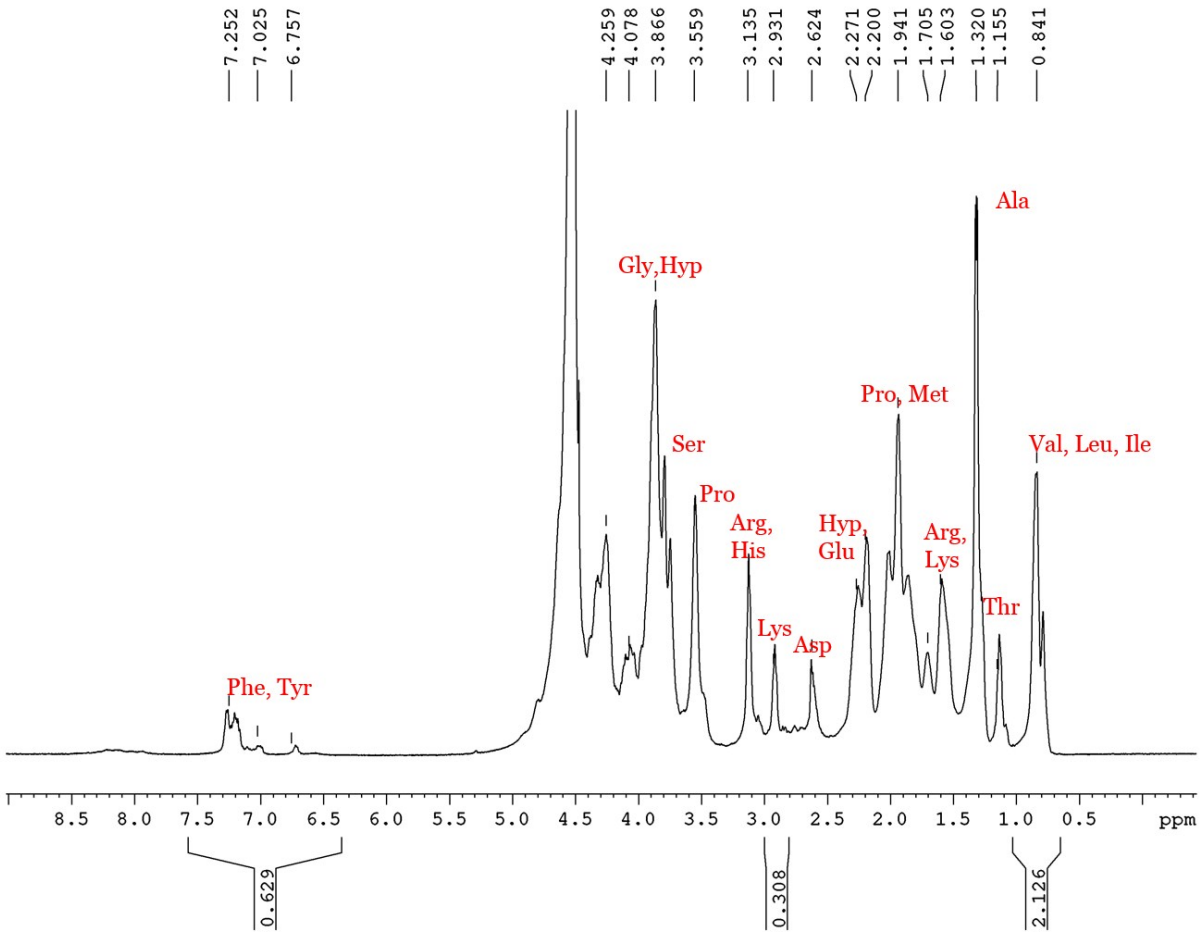

Figure S3. ^{1}H NMR spectrum of Native Fish gelatin. Location of the peaks according to Billiet et al.[2] We pay particular attention to 1) the methyl protons on Valine, Leucine, and Isoleucine at 0.84 ppm; 2) the ethylene protons attached to the carbon next to the primary amine on lysine at 2.93 ppm; and 3) the aromatic protons of phenylalanine and tyrosine at 7.25 ppm. These peaks can be used to quantify the conjugation efficiency to the gelatin macromer. The integrated peaks of the NMR matches well with the calculated proton concentration calculated above. For this study, we use the Val, Leu, Ile proton peak as an internal standard.

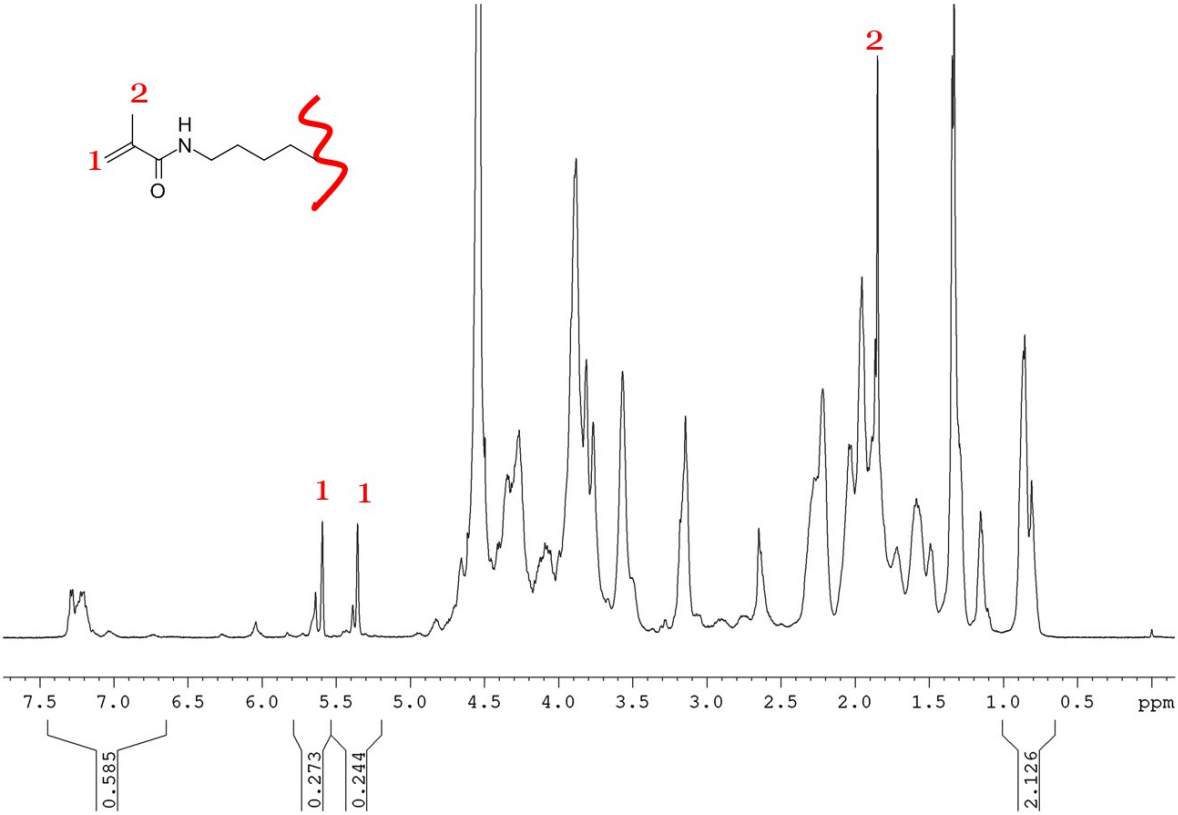

Figure S4. ^{1}H NMR spectrum of gelatin methacrylamide (GelMA) (**11**)

Amino Acid	Composition (mg/g)	Composition (mmol/g)	# NMR Protons	mmol/g protons	AA type	mmol/g Protons
Aspartic acid	38.9±4.59	0.292			Val, Ile, leu	2.124
Glutamic acid	71.7±1.08	0.488			lys	0.292
Serine	Not detected	0			tyr, phe	0.697
Glycine	308±12.1	4.107				
Histidine	Not detected	0				
Arginine	29.5±0.84	0.17				
Threonine	134±8.35	1.126				
Alanine	76.1±5.20	0.855				
Proline	Not detected	0				
Tyrosine	5.95±0.78	0.033	4	0.132		
Valine	17.7±1.60	0.151	6	0.906		
Methionine	14.2±2.03	0.095				
Cysteine	1.51±0.14	0.012				
Isoleucine	8.39±0.14	0.064	6	0.384		
Leucine	18.2±1.27	0.139	6	0.834		
Phenylalanine	18.6±0.51	0.113	5	0.565		
Lysine	21.3±2.21	0.146	2	0.292		
Tryptophan	Not detected	0				

Table S 1. Amino acid composition of Red Tilapia according to a previous study quantifying the amino acid composition of red tilapia (*Oreochromis nilotica*),[1] we calculate the molar amount of each amino acid per gram of gelatin. Once this data is known, we can calibrate the NMR spectrum to the expected molar amount of protons detected at each peak. In particular we calculate three specific peaks: 1) the methyl protons on Val (6 protons), Leu (6 protons), and Ileu (6 protons)located at 0.84 ppm; 2) the ethylene protons attached to the carbon next to the primary amine on lysine (2 protons) located at at 2.93 ppm; and 3) the aromatic protons of phe (5 protons) and tyr (4 protons) at 7.25 ppm. We can then calculate the mmol protons/g gelatin for these three respective regions (last column).

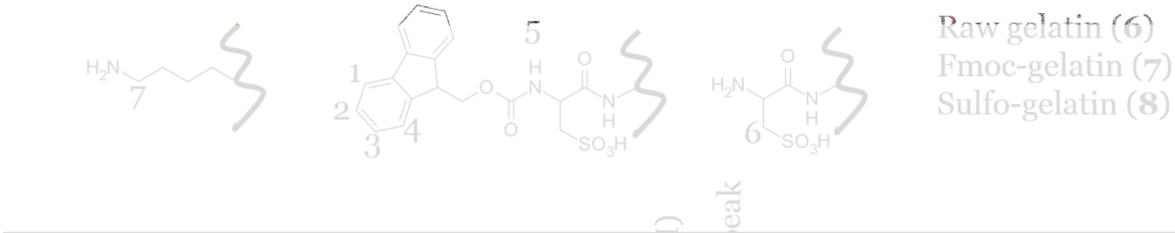

Figure S5. ^{1}H NMR spectra of native gelatin (**6**), Gelatin-Cys(O_3H)-NH-Fmoc (**7**), and deprotected Gelatin-Cys(O_3H)-NH_2 (**8**). In the native gelatin, we can see the ethylene proton peak corresponding to the protons on the carbon adjacent to the amine of the lysine. Once Fmoc-Cys(O_3H)-OH (**4**) is conjugated, this peak disappears and we find the proton peaks corresponding to the fmoc group. After deprotection of the fmoc group, we show that they peak corresponding to lysine is still not present and the peak corresponding to the cysteic acid appears. The fmoc proton peaks have disappeared, showing the successful deprotection.

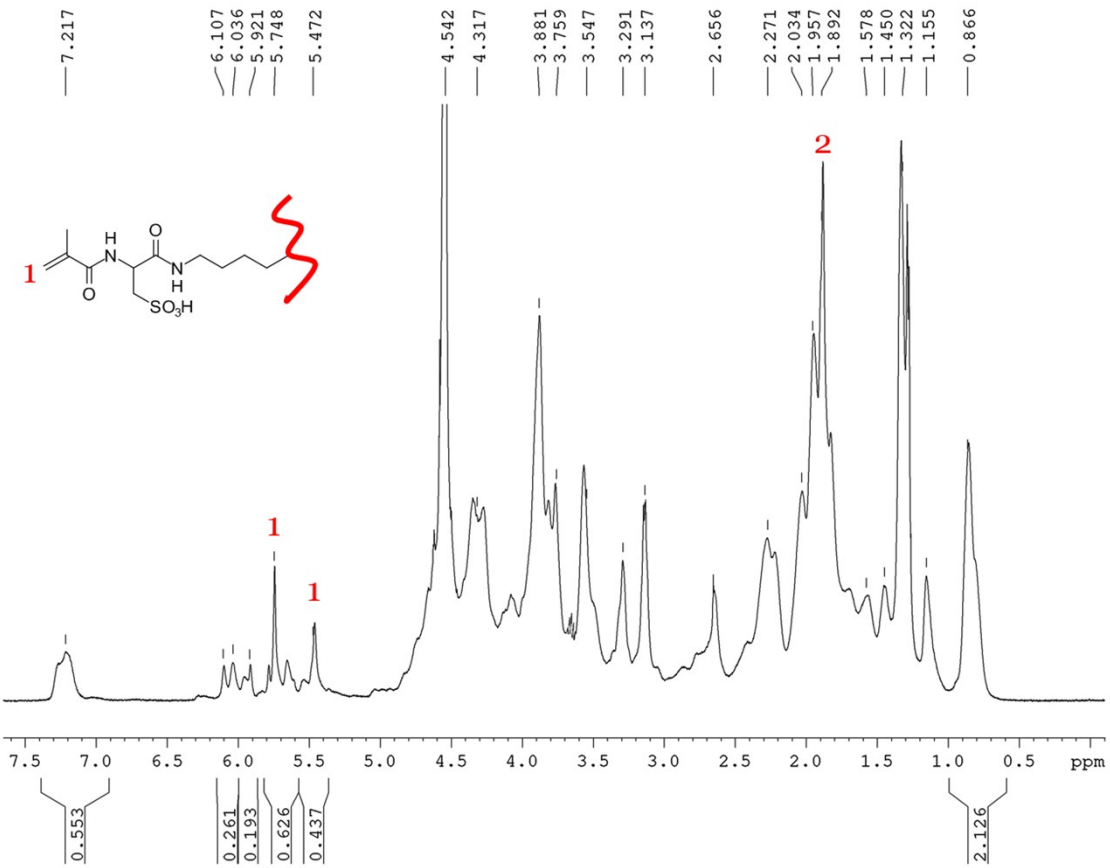

Figure S6. ^{1}H NMR spectrum of sulfo- gelatin methacrylamide (**12**). We notice how the protons on the methacrylamide peak shift now that the methacrylamide is attached to cysteic acid rather than lysine. We also find the presence of other methacryl protons around 6 ppm. These are likely methacryl groups attached to alcohol side chains in the gelatin (serine, threonine, and tyrosine) or possibly to histidine since we see an extra peak at 3.29 ppm.

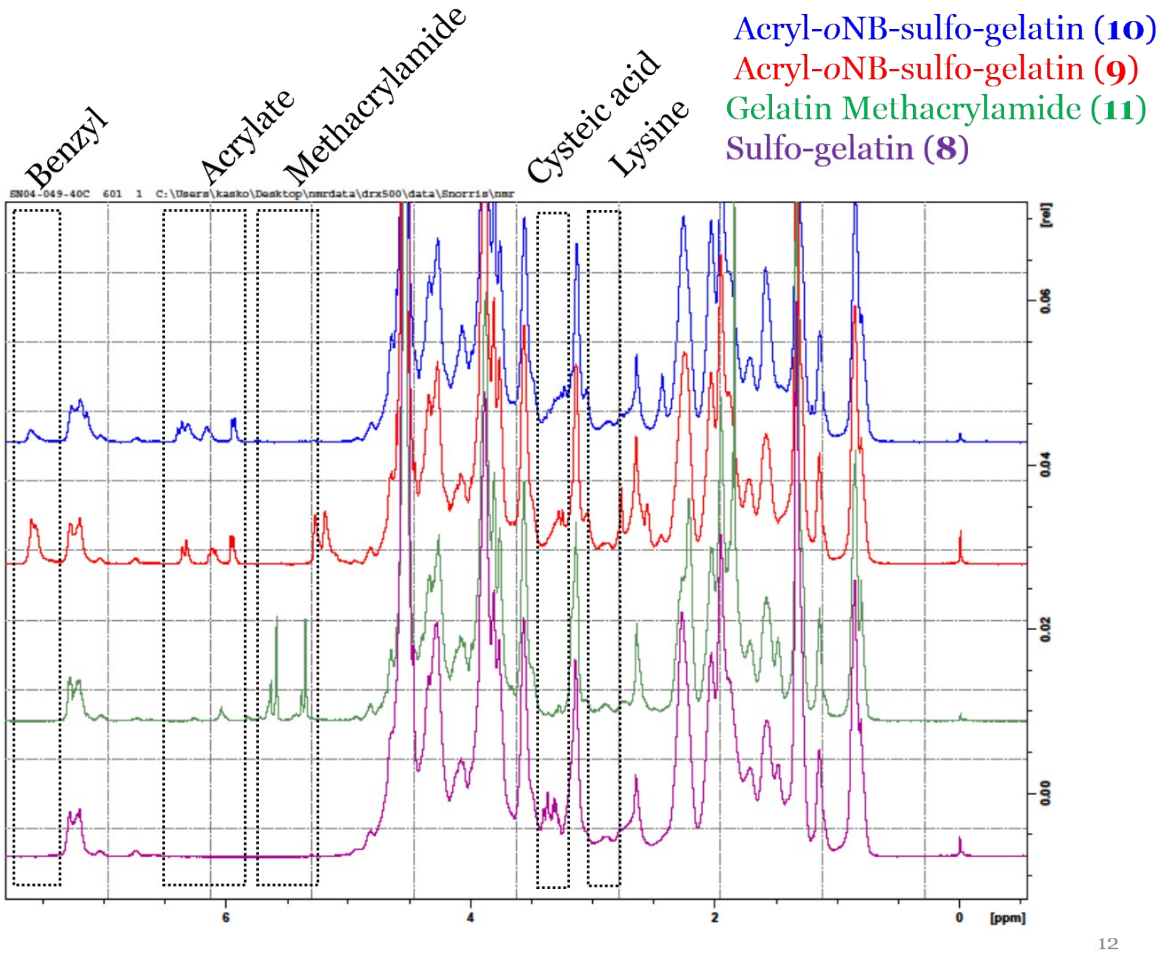

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Figure S7. Comparison of the ^{1}H NMR spectra of gelatin-Cys(O_3H)-NH_2 (**8**), gelatin methacrylamide (**11**) and the two acryl-*o*-NB-sulfo-gelatin samples (**9** & **10**). We pay special attention to the appearance and disappearance of several peaks corresponding to: 1) the benzyl peaks of the *o*-NB moieties; 2) the acrylate peaks of the *o*-NB moieties; 3) the methacrylamide peaks of GelMA; 4) the proton peaks on the cysteic acid; and 5) the lack of the lysine proton peaks on all samples.

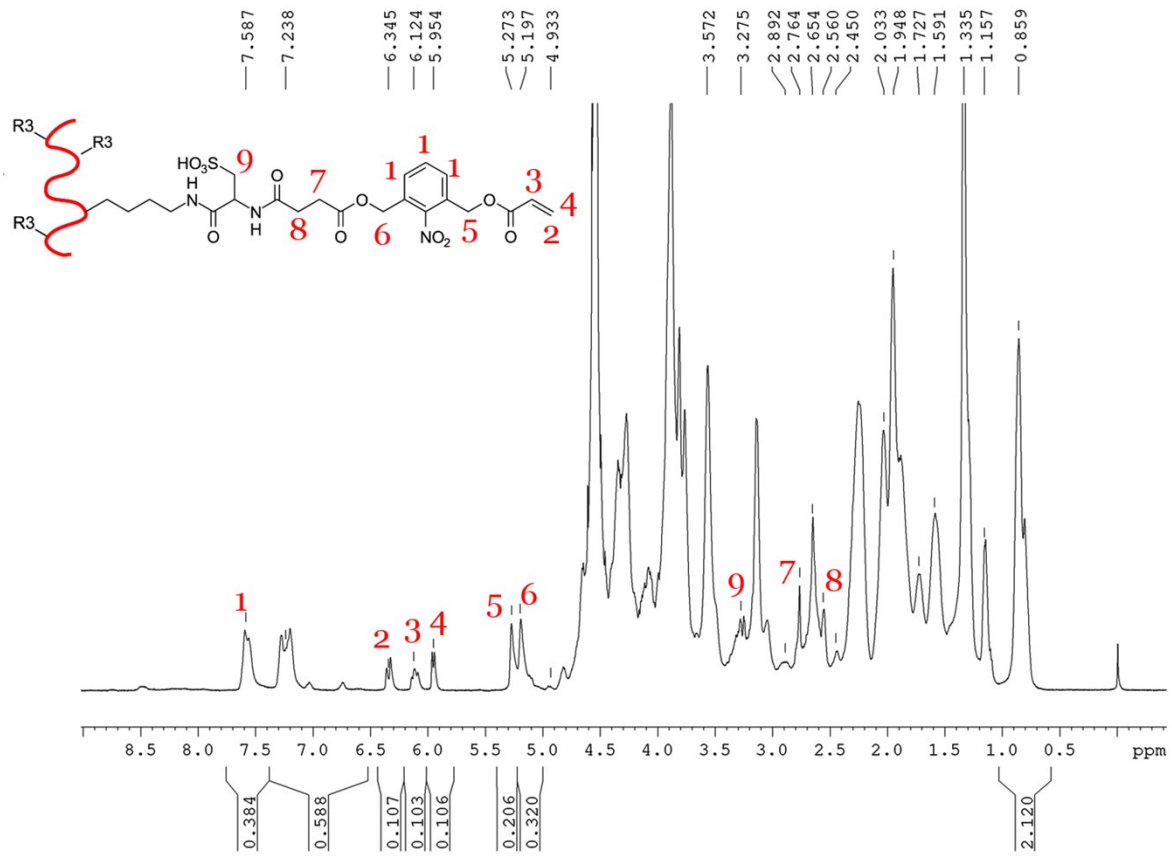

Figure S8. ^{1}H NMR spectrum of acryl-oNB-sulfo-gelatin (**9**)

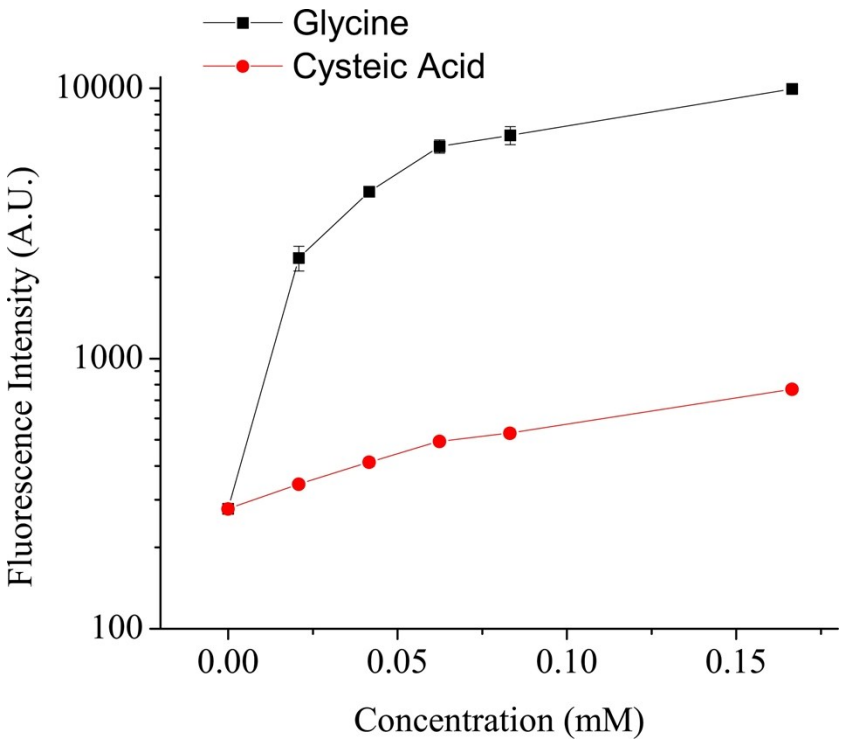

Figure S9. Standard curves for glycine and cysteic acid for the CBQCA assay. As noted above, we noticed that the primary amines of cysteic acid tend to react less readily than the primary amines of glycine. Thus, we produced a standard curve using cysteic acid and found that it reacted with the CBQCA assay orders of magnitude less than glycine. We paid special attention that the pH of the two amino acid solutions was the same. Thus, we recalibrated the calculation of primary amines remaining for the sulfo-gelatin (**8**) and the oNB-gelatin (**9**) using the cysteic acid standard curve.

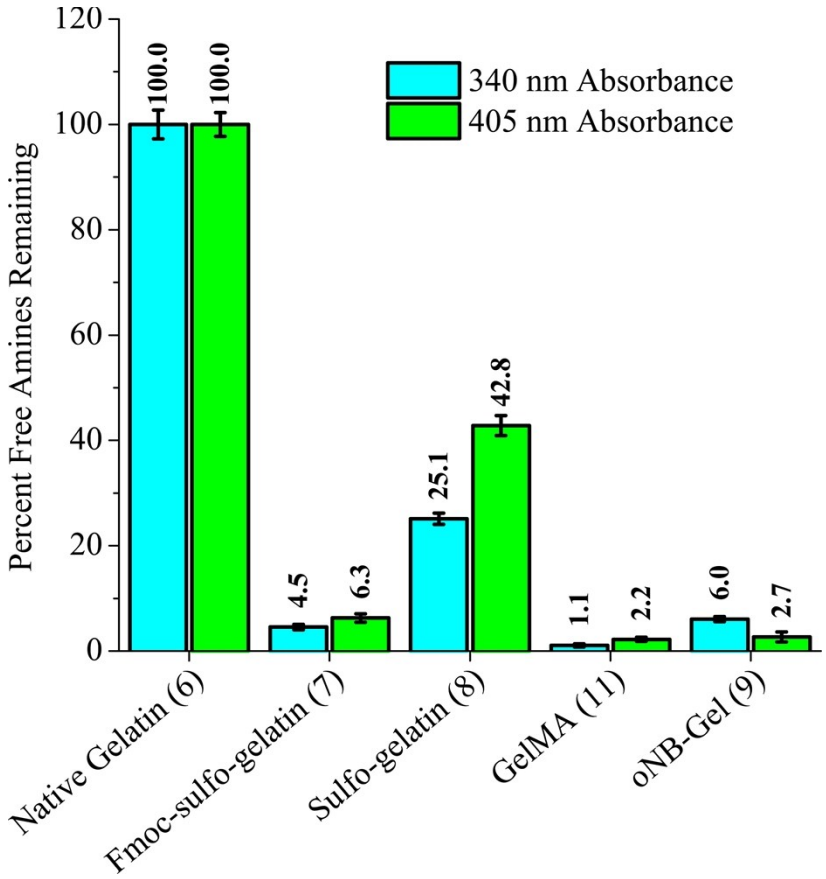

Figure S10. TNBS assay as measured using the absorbance of two different wavelengths of light. We found that if the reaction of the TNBS reagent when analyzed by the absorbance of 405 nm light (instead of 340 nm as suggested by the manufacturer), the sulfo-gelatin (**8**) showed a relatively higher signal, which could indicate that the chromogenic derivative of the assay is different when assaying cysteic acid residues. We do not believe that there is only a partial recovery of the amine group after Fmoc removal, but rather, the TNBS amine assay does not properly assay for amines on cysteic acid residues.

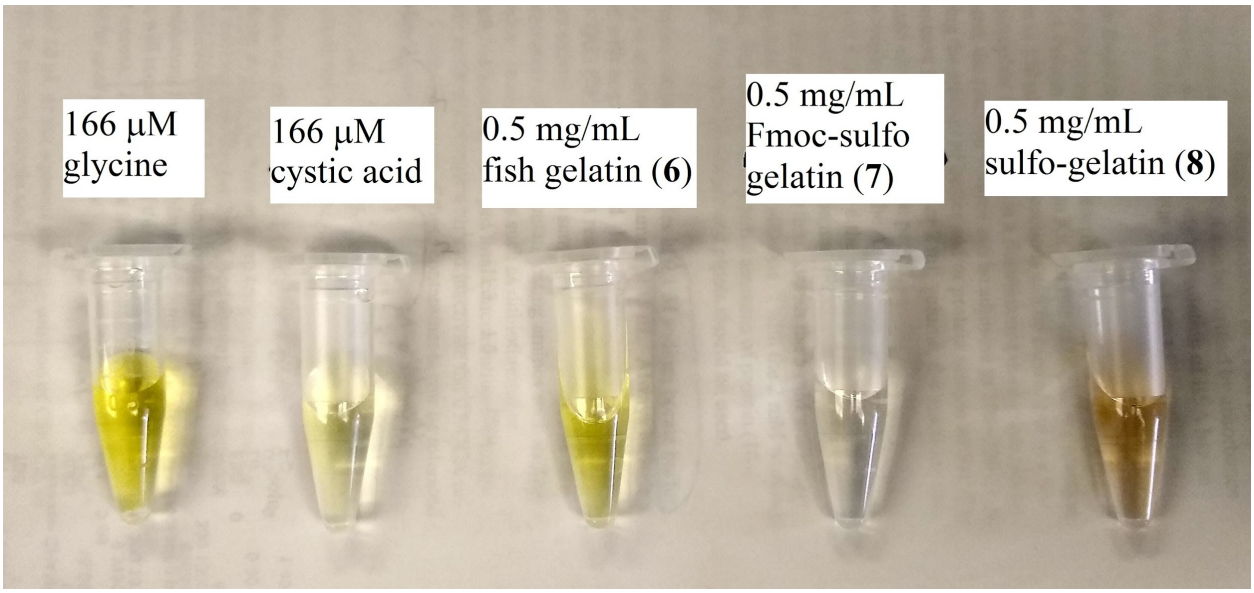

Figure S11. Images of TNBS assay solutions. During the TNBS assay, we noticed that the sulfo-gelatin (**8**) has a pinkish hue, as opposed to the yellow color of the glycine standards and the native fish gelatin (**6**). We have observed this difference in color across several independent experiments and multiple batches of sulfo-gelatin **(8)**. We believe that the conjugated cysteic acid moieties may interact with the TNBS assay differently than the primary amines of either gelatin's lysines or the primary amine on glycine (the typical/suggested source of primary amines for TNBS assay standard curves). This is supported by the above image showing the 166 μM cysteic acid sample, noticeably less saturated than the 166 μM glycine sample, even though they contain the same number of primary amines (the pH of the two solutions was the same).

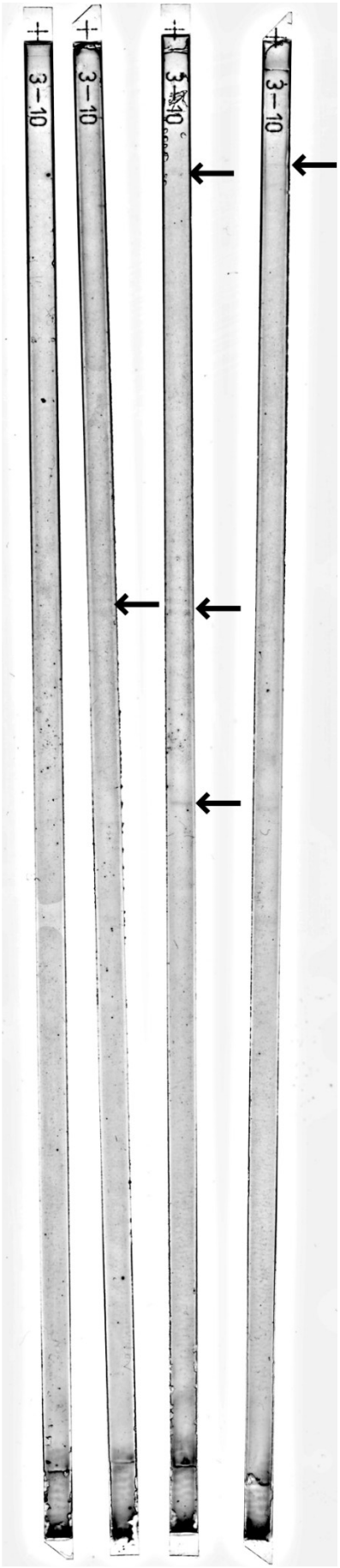

Figure S12. Iso Electric focusing gels. The right most gel is of the acryl-oNB-sulfo-gelatin (**9**) sample. The second from the right is the native fish gelatin (**6**). The second from the left is the Gelatin-Cys(O_3H)-NH-Fmoc (**7**) sample. The left most gel is of the deprotected Gelatin-Cys(O_3H)-NH_2 (**8**) sample. In the native fish gelatin, we find a prominent band at pH 6.6. This corresponds well to the pH range given by the manufacturer. There is a less prominent band at pH 5.7 which could correspond to different molecular weight gelatin strands, and another very faint band at pH 3.7, which we disregard. The gelatin-Cys(O_3H)-NH-Fmoc (**7**) sample has a faint peak at pH 5.6, which is very similar to the peak found in the native fish gelatin, which indicates that it could be an artifact. For the deprotected gelatin-Cys(O_3H)-NH_2 (**8**) sample we found no observable bands. In the acryl-oNB-sulfo-gelatin (**9**) sample we found possibly one faint band at pH 3.6, but this could again be an artifact since it also exists in the native gelatin sample. Altogether, this results suggests that addition of the cysteic acid moiety reduces the isoelectric point so strongly, that it is out of the range of this testing method. These isoelectric focusing strips had the lowest pH range we could find.

1 B. Jamilah and K. G. Harvinder, *Food Chem.*, 2002, **77**, 81–84.

2 T. Billiet, B. Van Gasse, E. Gevaert, M. Cornelissen, J. C. Martins and P. Dubruel, *Macromol. Biosci.*, 2013, **13**, 1531–45.